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UNCLAS STATE 204438
E.O. 11652:N/A
TAGS: TGEN, UR
SUBJECT: S&T: MICROBIOLOGY WORKING GROUP MEETING
        SEPT. 21-30
REF: MOSCOW 11068
FOR SCICOUNS
LEISE REQUESTS EMBASSY PASS FOLLOWING TO SHENDEREY:
1. USDEL WILL CONSIST OF:
JOSHUA M. LEISE, NSF, DELEGATION LEADER
ARTHUR E. HUMPHREY, UNIVERSITY OF PENNSYLVANIA
DANIEL I. C. WANG, MIT
EDMUND FIELD, PRIVATE CONSULTANT
GEORGE TSAO, PURDUE UNIVERSITY
ROBERT K. MORTIMER, UNIVERSITY OF CALIFORNIA, BERKELEY
MRS. LEISE WILL ACCOMPANY.
2. USDEL WILL ARRIVE SEPT. 21 AT 1215 VIA PA 44. LEISES
WILL DEPART OCT. 5 AFTER JOINT COMMISSION MEETING;
HUMPHREY, WANG, FIELD AND TSAO WILL LEAVE SEPT. 30.
MORTIMER WISHES TO LEAVE SEPT. 25 OR 27.
3. SITE VISITS:
A) LEISE WISHES TO DISCUSS FURTHER WITH SHENDEREY
ANTICIPATED VISIT TO SINGLE CELL PROTEIN PLANT AT GORKY.
B) EARLIER SOVIET DELEGATION DESCRIPTION OF UFA PLANT AS
HAVING OLD TECHNOLOGY PLUS DISTANCE BETWEEN MOSCOW AND
UFA MAKES VISIT THERE OF LESS INTEREST THAN VISIT TO
KIRISHEY NEAR LENINGRAD, WHERE, WE UNDERSTAND FROM
SOVIET PRESS, SCP PLANT USING HYDROCARBONS AND WOOD
HYDROLYSIS AS SCP SUBSTRATES AND ENZYME HYDROLYSIS PLANT
INVOLVED WITH HYDROLYSIS OF WOOD ARE LOCATED.
C) USDEL ALSO WISHES VISIT NATURAL GAS PLANT OF
INSTITUTE OF GAS TECHNOLOGY AT KIEV, BEING USED FOR
FOOD PRODUCTION; INSTITUTE OF CELLULOSE HYDROLYSISYEAST
GENETICS INSTITUTE AT LENINGRAD; AND BACH INSTITUTE OF
BIOCHEMISTRY AND ALL UNION SCIENTIFIC RESEARCH INSTITUTE
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ON PROTEIN SYNTHESIS IN MOSCOW.
[) IF IT IS IMPRACTICAL FOR ENTIRE DELEGATION TO VISIT
ALL OF ABOVE SITES, HUMPHREY, WANG, FIELD AND LEISE
COULD VISIT KIRISHEY WHILE MORTIMER AND TSAO VISITED
TWO INSTITUTES IN LENINGRAD. PREFER LENINGRAD INSTITUTES
BE SCHEDULED RIGHT AFTER WG MEETING TO PERMIT MORTIMER
TO LEAVE NO LATER THAN SEPT. 27. WHILE HUMPHREY, WANG
AND FIELD VISIT KIEV, LEISE AND TSAO COULD VISIT TWO
MOSCOW INSTITUTES, ALTHOUGH ENTIRE DELEGATION WOULD LIKE
TO VISIT TWO MOSCOW INSTITUTES.
4. WE PROPOSE THAT WILLIAN B. ARMIGER ATTEND WG MEETING
AS OBSERVER ON SEPT. 22 AND 23 AND THEN MAKE FOLLOWING
VISITS:
SEPT. 24-26 - INSTITUTE FOR BIOCHEMISTRY AND PHYSIOLOGY
OF MICROORGANISMS, PUSHINO NA OKA, DR. YEROSHIN;
SEPT. 27-28 - LENINGRAD (PERSONAL);
SEPT. 29-OCT. 7 - KAZAN INSTITUTE OF CHEMICAL TECHNOLOGY,
DR. YENIKEYEV;
OCT. 8-10 - INSTITUTE OF BIOTECHNOLOGY AND INSTITUTE FOR
PROTEIN SYNTHESIS, MOSCOW, DR. POSTNIKOV.
HE WOULD ARRIVE WITH DELEGATION SEPT. 21 AND DEPART OCT.
11. YOU WILL RECALL WE SUGGESTED HE VISIT USSR JUNE 15-
JULY 15, BUT KOTOV TOLD US AN OCTOBER VISIT WOULD BE
PREFERABLE.
5. WOULD APPRECIATE SOVIET SIDE MAKING ABOVE SITE VISIT
ARRANGEMENTS AND AUTHORIZING SOVEMB WASHINGTON TO ISSUE
VISAS. INGERSOLL

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To the Co chairman of the Joint
U.S./U.S.S.R. Working Group
on Production of Substances by
Microbial Means

STATINTL

Dr. J. M. Leise
Dr. E.R. Shenderey

R E P O R T

ON THE JOINT U.S./U.S.S.R. CONFERENCE ON "DATA
ACQUISITION AND PROCESSING FOR LABORATORY AND
INDUSTRIAL MEASUREMENTS IN FERMENTATION PROCESSES"
HELD AT THE UNIVERSITY OF PENNSYLVANIA, PHILADELPHIA,
PENNSYLVANIA, AUGUST 12-15, 1975.

In accordance with the June 19, 1974 agreement of the Joint US/USSR
Working Group on Production of Substances by Microbial Means reached in
Washington, D.C., a joint conference on "Data Acquisition and Processing
for Laboratory and Industrial Measurements in Fermentation Processes"
(Project 2, Task 1.1) was held at the University of Pennsylvania,
Philadelphia, Pa., U.S.A. during the period August 12-14, 1975.
Attending the Conference in an official capacity were 6 Soviet and
14 American official delegates. In addition there were 16 observers from
various U.S. Institutions and Companies. (The list of Attendees from both
sides is given in Appendix I). During the Conference 18 formal papers
were presented - 5 papers by the Soviet delegates and 13 papers by the
American delegates. (The conference format is given in Appendix II).

On August 15 the official delegates of both sides discussed the
details of the tasks of cooperation under Project 2. The report to the
Co-chairman of the Joint U.S./U.S.S.R. Working Group on the visit to the
U.S.S.R. of Professor L. Erickson of Kansas State University in May and
June of this year formed the basis of the discussion.

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As a result of these discussions each side plans to submit a proposal on the details of future working plans for Project 2 for consideration and possible adaptation by the Joint Working Group at its next meeting in Moscow in September 1975.

The Soviet Delegation also visited the Laboratories at the University of Pennsylvania, U.S. Army Natick Development Center, Massachusetts Institute of Technology, and the Leeds & Northrup, Foxboro, and Fermentation Design Companies. (The schedule details are given in Appendix III).

General Comments

The Conference proved to be a useful mechanism for the exchange of information on the progress in the area of instrumentation, computerization, and modeling of fermentations. At the conclusion of the Conference a series of summary statements were evolved regarding the state of the art and indicated future research needs in this area of cooperation.

During the Conference a number of reports were made on research and development of new instrumentation for monitoring fermentations. The emphasis of these reports were on the need for continuing development of instrumentation to measure biomass and substrate concentration, particle size distribution, (microbial cells, hydrocarbon droplets, etc.) and parameters of microbial physiological activity and culture media.

Progress was reported in the area of mathematical modeling of both pure and mixed cultures grown on water soluble and insoluble substrates, such as hydrocarbon and cellulose, for the production of single cell protein. Reports from both sides emphasized the need for experimental verification and further refinement of the models.

In the area of computer-aided data acquisition and analysis, sub-
stantial progress in both countries is occurring. This progress should

make possible on-line control and dynamic optimization of fermentation

processes. Systems for the control of the physical environment, such

as temperature, antifoam, etc., are well developed. Systems for control

of the physiological state of the microorganisms have not generally been

realized.

There has been little practical realization in the area of optimi--
zation. However, the group was encouraged by the reports of activity

from both countries concerning optimal fermentor design and on-line

optimization. From these limited reports the attendees at the conference

were generally optimistic over the future prospects for on-line fermentation

process optimization.

A.   SUMMARIES OF MAIN TOPICS

Measurement

In the session on Measurement, six (6) papers were presented by the Symposium participants. These papers focused on the problems of measurement of biomass, and substrate concentration.

A new method for measuring biomass was presented by V. Babkin of the Institute of Biotechnology. His paper discussed measurement of biomass by measuring the cytochrome c content of Candida yeast grown on paraffins. Techniques of compensation for the influence of culture medium turbidity, light source instability and coefficients of transmission of the photocell were presented and their effects on the accuracy of the measurement were discussed.

D. Zabriskie of the University of Pennsylvania presented a paper on the utilization of fluorometry, kjeldahl nitrogen, potassium and oxygen measurements for measuring biomass concentrations. This paper discussed methods for measuring organic nitrogen which can be used to determine cell mass in a broad class of fermentations including the cellulose fermentation. With this procedure being used as a reliable laboratory procedure, a number of on-line estimation instruments and techniques are being developed. These include estimates based on culture fluorescence and component balancing. The components studied include carbon, oxygen, and potassium. These estimates will provide valuable information to manual or computer mediated fermentation control.

The application of the conductometric method of dispersion analysis for the analysis of microbial populations was presented by V.V. Mitjaev of the Institute for Biotechnology. This paper included theoretical and

and experimental studies on this technique and discussed its application to the microbial industry. Consideration was given to the problems of optimal orifice diameter, coincidence error measurement, and compensation for error from changes in electrolyte concentration.

W. M. Krebs from Instrumentation Laboratory, Inc., presented a paper on a steam sterilizeable polarographic electrode for measurement of dissolved oxygen. These electrodes have a fast response time with excellent linearity and exhibit virtually no sensitivity to sample rheology. The background current of the device is very low. This fact coupled with its excellent linearity, means that the device can be calibrated at one point, in air, even for low oxygen analyses and is very useful in monitoring fermentation.

Two indirect methods of assessing the cell concentration were presented by J. Swartz of M.I.T. One method examined the measurement of heat of fermentation and its correlation with cell mass production. A dynamic calorimetric technique was employed to measure the rate of heat evolution during the fermentation.

A second approach presented was the use of broth viscosity to determine cell concentration. The pressure drop through a thin channel was shown to be proportional to the culture viscosity. Using pressure transducers a continuous viscometer was built to evaluate this approach.

O. Komarov of the Institute of Biotechnology presented a paper on methods to measure the concentration of n-alkanes and inorganic phosphate in culture media. The problems of development of laboratory methods and equipment were considered. Results from a laboratory system were presented to demonstrate the use of this technique.

INDICATED RESEARCH NEEDS

The application of computers to fermentation processes requires the use of reliable sensors for measurement of biomass and substrate concentrations. Currently, there are no sensors commerically available which are suitable for continuous biomass concentration measurement in industrial scale fermentors. However, some promising techniques were presented at the symposium. There is a need to develop reliable and accurate direct methods for measuring biomass concentration. In addition to direct methods, indirect methods should be evaluated on industrial scale fermentations. Further development of methods for measuring population structure and their application for large scale systems should be examined.

It is necessary to develop sensors to measure concentrations of the most important components of the culture medium, such as cellulose and hydrocarbon substrates.

Also, development is required for techniques and devices for preparation of degassed medium samples for monitoring purposes.

B. ANALYSIS AND INTERPRETATION

In the session on "Analysis and Interpretation" Dr. Charles L. Cooney presented a paper on the computer-aided fermentation for the monitoring and diagnostics of fermentation systems.

In this paper it was indicated that the number of sensors available for monitoring fermentations is rather limited and sensors to measure the most important parameters such as cell biomass and metabolic product concentration have not been totally perfected. Therefore, it is necessary to deduce the values of these parameters from other measurements. Instruments which are commercially available enable one to measure pH, temperature, dissolved oxygen, gaseous oxygen and carbon dioxide, and air flow rate. In addition, by means of load cells, it is possible to monitor nutrient flow rate and acid or alkalie flow rates. Using differential thermometry, heat production during fermentation can also be measured. From these data and the use of material and energy balances, it is possible to continuously determine the concentration of biomass and the conversion yield of substrate to biomass.

Having established the ability to indirectly assess cell biomass and conversion yield, it is possible to use the computer on-line to detect deviations from optimal conditions. To achieve this, diagnostic strategies were developed to identify possible reasons for a decline in productivity and/or cell yield. These strategies are based upon both steady-state and transient analyses of the fermentation. The application of computer-aided diagnostic analysis to the production of yeast single-cell protein from glucose was discussed using the techniques outlined above.

INDICATED RESEARCH NEEDS

The indirect technique for determining biomass concentration appears to be an useful method for predicting biomass formation during fermentation. The use of this method for determining cell yield of biomass and productivity is again encouraging for computer-aided fermentations. However, the validity of this method assumes an accurate knowledge of the stoichiometric coefficients for the energy and material balances. In addition, these stoichiometric coefficients have been assumed to be constant during the time course of batch and continuous fermentations. It appears that the validity of assuming constant stoichiometric coefficients should be carefully evaluated. The overall accuracy of the method should also be estimated.

The ability to determine the metabolic product concentrations is shown to be useful for diagnostic purposes and for optimization of biomass yield for th production of single-cell protein. There is a need for research in conjunction with direct measurement techniques of these product concentrations during fermentation.

The indirect techniques of determining biomass concentration and metabolic product concentration are highly dependent on the reliability and accuracy of the available instruments. One must therefore assess the influence of the reliability and accuracy of these instruments on these indirect techniques.

C. MODELING

Modeling is important in achieving the goals of the cooperative research. At this meeting, papers were presented on the modeling of four phase fermentation processes (hydrocarbon and cellulose fermentations) and mixed cultures on mixed substrates. An approach to development of a general mathematical model applicable for the purposes of optimal design and scale-up of multiphase fermentation processes was introduced. This approach is based upon decomposition of the process into a hierarchy of quasi-closed subsystems. Research on the use of material and energy balances in fermentation process identification was presented and the group method of data handling for on-line model building was introduced and demonstrated.

Several significant advances were reported:

A new model for cellulose hydrolysis and growth of Thermoactinomyces was described. This model will be useful in the production of single cell protein from cellulose.

The pathways for oxygen transfer in hydrocarbon fermentations were presented and it was shown that cells adsorbed to air bubbles utilize oxygen at a much slower rate than the unhindered rate of oxygen transfer to the continuous phase. Cells adsorbed to air bubbles may hinder oxygen transfer to the continuous phase and reduce the rate of oxygen transfer from the air bubbles. It was shown that the residence time distribution of air bubbles is important in the design of air-lift and recirculation fermentors.

Mathematical models and simulation have been used to interpret experimental results with mixed cultures on mixed substrates. Culture instabilities of the mixed population were simulated and important variables were identified. The results of research on mixed cultures will be useful in the design of large scale mixed culture processes such as in single cell protein production.

Advances in material and energy balance models were reported in several papers. Models of this type are particularly useful in process identification and control, now that highly instrumented computer coupled fermentation systems are available. The group method of data handling for on-line modeling will be very useful in future research.

INDICATED RESEARCH NEEDS

Process identification research needs to continue because model building research is very important for exploiting the usefulness of laboratory and pilot plant highly instrumented computer coupled fermentation systems, and models are important in the computer-aided synthesis of optimal fermentation processes. For complex processes such as the hydrocarbon fermentation, cellulose fermentation, and mixed culture systems, both experimental and theoretical work are needed. Experimental and theoretical research tasks which are necessary in development of a general mathematical model of multi-phase fermentation processes were formulated previously[1] and reviewed at the conference.

Further oxygen and carbon substrate transport research is needed because these processes are particularly important to growth and process economics in hydrocarbon fermentations. In order to adequately model oxygen transport, a good general model for hydrocarbon fermentations is needed because bio-chemical factors affect oxygen transfer and the oxygen transfer pathways. Much additional research is needed to develop a general model for hydrocarbon fermentations.

There is a need for research on multiphase flow and mixing in cultures with two liquid phases. The effect of equipment design and operating con-ditions on transport and growth needs further investigation. Research on

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[1]See Appendix I of the report on the visit in the U.S.S.R. of Professor Larry E. Erickson.

the dynamic ... prediction of the growth behavior of individual cells under various local environmental conditions is needed.

In the cellulose fermentation, the lag phase is not adequately modeled at present. Additional model building is needed for synthesis of optimal processes. In multiphase type of fermentations such as hydrocarbon and cellulose, there is a need for an understanding of fundamental processes in order to generalize the models such that they will be useful from species to species and from one size and type of equipment to the next.

Mixed culture modeling is just beginning. Further work with the present system (Hansenula polymorpha and Torulopsis candida) as well as work with other systems is needed. The hydrocarbon and cellulose research can be extended to mixed cultures growing on mixed substrates. Research experience with model systems will be helpful in carrying out studies with these more complex systems.

There is a need to incorporate the best model building and process identification techniques while carrying out modeling research. Full use needs to be made of material and energy balances in fermentation research and in industrial operation and control. The development of computer software, such as a computer-aided design system which will be useful in model building, simulation and process synthesis is needed.

D.   INTERFACING AND CONTROL

Five papers were presented at this session. Dr. J.M. Nystrom, (U.S. Army Natick Development Center) presented the paper on "Equipment and Instrumentation Requirements for Cellulose/Cellulase Processing".

He felt that bio-processes utilizing insoluble substrates present the bio-engineer with a variety of problems, among them the handling and transport of solids and slurries, effective oxygen transfer, and estimation of cell mass in the presence of insolubles. The production of cellulase enzymes in submerged culture and the continuous enzymatic hyrdolysis of waste cellulose are two such troublesome processes. The selection of equipment, instrumentation, and operating parameters are all of equal importance as is the development of appropriate systems and models for data reduction.

Dr. N.N. Postnikov, (Institute of Biotechnology) presented the paper on "Computer Interfacing in Fermentation Processes" in which he described three special electronic devices for computer interfacing. At the present time, two prototype devices have been built. The first is for the conductometric sensor, and the second prototype is for the hydrocarbon concentration sensor. The operation of the third device, which is used for data compression, has been simulated on a computer with good results.

Dr. M.C. Beaverstock, (The Foxboro Co.) presented the paper on "Common Principles of Plant-Scale Computer Control". He pointed out that computer controlled plants in differnet industries contain many common principles of operation. All systems require hardware for primary measurements, signal conditioning, interfacing, and computation. They also require software for inout/output, control algorithms, process models, alarms, displays, program development, and system operation. Therefore a properly designed computer controlled system consists of many independent hardware and software modules that can be easily modified for use with any process.

Dr. J.D. Wilson, (ABEC, Inc. Allentown) presented a paper on "Planning a Computer Coupled Fermentation Production Plant". He suggested that the ultimate test of a plant design is the unit cost of the product produced. Among the current factors influencing fermentation costs are fluctuating and upward trending raw material prices, much higher energy costs, and increased price competition as some products shift from proprietary to generic status. The commercial development of new processes as well as expanded production capacity for existing ones will likely depend on attractive investment return based on anticipated lower unit costs.

Dr. L.K. Nyiri (Fermentation Design) presented the paper on "Application of Computers to the Analysis and Control of Microbiological Processes", in which he pointed out that digital computers have proven useful for acquisition of process data and definition of process activity. Computers have made it possible to perform pulse testing of cultures, to alter environmental variables and to observe the responses in changes of the culture's rheological and physiological conditions. Analysis of data resulted in development of a control technique which performs the control of individual environmental variables on the basis of the physiological condition of the culture. The environmental variables are changed in an interactive way. The application of such a control technique in a molasses based SCP production resulted in enhanced yield and productivity of C. utilis cell mass.

According to Section 5.3 of the working program on "Engineering Research and Development of Equipment and Techniques, Computerized Simulation, Design, and Control of Processes for Microbial Technology" our goal is the "Demonstration in the U.S.S.R. of a Computer Controlled Process for Producing Single Cell Protein". In order to meet this objective, it is recommended that a highly instrumented computer coupled fermentation system employing the latest concepts and the most advanced equipment should be developed. This system would be used in a cooperative effort for testing new sensors, evaluating fermentation models, and implementing new control strategies. Since many of the system concepts can be applied to a study a variety of fermentations, it is recommended that the cooperative effort for evaluating the fermentation system be expanded to include work directed toward single cell protein from cellulose.

E.   OPTIMIZATION

The paper presented in this session discussed the principle and application of the GMDH algorithm (the Group Method of Data Handling) to on-line optimization in fermentation systems. The GMDH algorithm is based on the principle of heuristic self-organization and is a new technique for mathematical prediction of the behavior of highly complex systems such as multiphase fermentations. The GMDH implements a truncated Kolmogorov-Gabor polynomial in the form of a multilayer system of the perception type. Data are partitioned into a training subset and a testing subset; the former is used in conjunction with a newly developed iterative random search procedure to obtain coefficient values that are globally optimum while the latter is used to determine the optimum degree of complexity of the model. The algorithm was demonstrated by predicting cell concentration using oxygen uptake data for a hydrocarbon fermentation.

INDICATED RESEARCH NEEDS

1.   Development of Inexpensive but Reliable Hierarchial Process
     Control Systems.

By carefully exploiting the capability of microprocessors as well as mini and macrocomputers, we should be able to develop relatively inexpensive but reliable hierarchal process control systems. Efficient forecasting techniques must be developed in conjunction with such control systems.

2.   Development of a Unified Approach to Fermentation Process
     Synthesis.

Proper coordination among major component activities of the process synthesis is still lacking. It appears to be highly desirable to establish a unified approach or approaches to biochemical process synthesis.

3.   Development of Easy-to-Use Global Optimization Techniques.

The morphology of fermentation processes are usually very complex, and thus the task of optimizing such a system when cast in the form of a general non-linear program, is often extremely formidable. It is desirable to develop global optimization

methods which are simple, have rapid convergence characteristics, and excellent numerical stability, and are suitable for on-line optimization of biochemical processes. Such optimization techniques should also be useful for estimating parameters of models for biochemical systems.

4. Development of Reliable Computer Softwares for Simulation of Dynamic Biochemical Processes.

An extremely reliable subroutine for solving second order partial differential equations has been developed recently. This opens the door for the possibility of writing a general purpose computer subroutine for simulating dynamic biochemical processes.
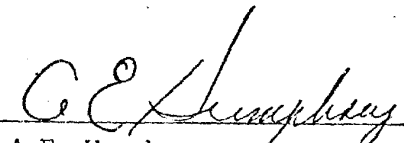
RECOMMENDATIONS

In view of the fact that this conference proved to be a valuable mechanism for information exchange and was an important vehicle to coordinate cooperation on the joint tasks, we recommend that the proceedings of the conference be published, and that such conferences be continued on an annual basis as part of the activity of Project-2 under the Joint Working Group agreement on cooperation in the areas of "Production of Substances by Microbial Means".
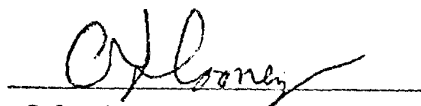
As a result of discussion, the Project Coordinators would like to specifically recommend amendment of the June 1974 agreement as follows:

1. Project-2 Conferences should be planned on an annual basis. The place of the Conferences should alternate between the two countries. With reference to publication of the proceedings, when Conferences are held in the U.S., the American Society for Microbiology would assume responsibility for publication of the proceedings, both in English and Russian. When conferences are held in the U.S.S.R., the Main Board for the Microbiological Industry would assume this responsibility.

2. The next planned conference should be held in the U.S.S.R. in 1976. Its title should be modified to read "Mechanisms and Kinetics of Uptake and Utilization by Microorganisms for Substrates of Low Solubility".

3. Inclusion of cellulose fermentations under the programs in Project-2, Task 2, and changing the title of Task 2 to read "Investigation of mass, momentum, and heat transfer in heterogeneous gas-liquid-liquid and gas-liquid-solid types of culture conditions as well as kinetics and biochemical mechanism of hydrocarbon-uptake and of cellulose utilization by microorganisms".

We would also like to recommend that exchange of personnel at the junior and senior research level commence.

Project 2 (two) Coordinators
U.S. Side

Project 2 (two) Coordinators
U.S.S.R. Side

_____
A.E. Humphrey

_____
S.G. Yenikeyev

_____
C.L. Cooney

_____
N.N. Postinikov

APPENDICES

U.S.S.R. PARTICIPANTS

1.	S. Yenikeyev	KICT, KAZAN
2.	N. N. Postnikov	All Union Institute for Biotechnology of the Main Board for Microbiological Industry
3.	O. B. Komarov	
4.	V. V. Mitjaev	
5.	V. J. Babkin	
6.	V. I. Seriogin	Main Board for Microbiological Industry

U. S. PARTICIPANTS

1.	A. E. Humphrey	University of Pennsylvania
2.	D. Zabriskie	University of Pennsylvania
3.	W. Armiger	University of Pennsylvania
4.	L. Erickson	Kansas State University
5.	L. T. Fan	Kansas State University
6.	J. Swartz	Mass. Inst. Tech.
7.	C. Cooney	Mass. Inst. Tech.
8.	D. Wang	Mass. Inst. Tech.
9.	J. Nystrom	Natick Laboratories
10.	L. K. Nyiri	Fermentation Design
11.	J. D. Wilson	ABEC, Inc.
12.	M. Beaverstock	Foxboro, Inc.
13.	T. Bay	Leeds & Northrup Company
14.	W. M. Krebs	Instrumentation Laboratories

OBSERVERS

1.	J. Leise	National Science Foundation (Chairman - U.S. Working Group)
2.	R. D. Watkins	American Society of Microbiologists
3.	J. Dobson	Foxboro, Inc.
4.	I. Haddon	Instrumentation Laboratories
5.	L. Evans	Mass. Inst. Tech.
6.	H. Wang	Mass. Inst. Tech.
7.	E. Lee	University of Pennsylvania
8.	T. Ladish	University of Pennsylvania
9.	A. Moreira	University of Pennsylvania
10.	Y. Lee	University of Pennsylvania
11.	R. Hatch	University of Maryland
12.	R. Wilcox	Mass. Inst. Tech.
13.	H. Taguchi	University of Pennsylvania(Visit. Prof.)
14.	H. Nakahara	Kansas State University (Visit. Prof.)
15.	C. Krishnaswami	Fermentation Design
16.	H. Bungay	Worthington Biochemicals, Inc.

U.S./U.S.S.R. SEMINAR ON

DATA ACQUISITION AND PROCESSING FOR LABORATORY AND INDUSTRIAL MEASUREMENTS I
FERMENTATION PROCESSES

AUGUST 12-14, 1975, UNIVERSITY OF PENNSYLVANIA

INTRODUCTORY PAPERS

1. ON THE PROBLEM OF MODELING OF FERMENTATION PROCESSES AND
   RESEARCH TASKS ON PROJECT "2"
   by S. Yenikeyev (Inst. Chem. Technol., Kazan)

2. RATIONALE AND PROBLEMS IN THE USE OF COMPUTER-COUPLED FERMENTATION
   SYSTEMS *by A. E. Humphrey (Univ. of Penna., Philadelphia)*

MAIN TOPICS FOR DISCUSSION

A. MEASUREMENT

1. BIOMASS CONCENTRATION MEASUREMENT
   by V. Babkin (Inst. Biotechnology, Moscow)

2. UTILIZATION OF FLUOROMETRY, KJELDAHL, POTASSIUM, AND OXYGEN
   MEASUREMENTS FOR ESTIMATING BIOMASS CONCENTRATIONS
   by D. Zabriskie (Univ. of Penna., Philadelphia)

3. CONDUCTOMETRIC COUNTERS OF PARTICLES FOR ANALYSIS OF MICROBIAL
   POPULATION *by V. Mitjaev (Inst. Biotechnology, Moscow)*

4. UTILIZATION OF THERMAL AND VISCOMETRIC METHODS FOR BIOMASS
   ESTIMATIONS *by J. Swartz, H. Wang, and C. L. Cooney
   (Mass. Inst. Tech., Cambridge)*

5. DETERMINATION OF n-ALKANES AND INORGANIC PHOSPHATE CONCENTRATIONS
   IN CULTURE MEDIA *by O. Komarov (Inst. Biotechnology, Moscow)*

B. ANALYSIS AND INTERPRETATION

1. COMPUTER-AIDED MONITORING AND DIAGNOSTICS *by C. Cooney, H. Wang,
   and D. I.-C. Wang (Mass. Inst. Tech., Cambridge)*

C. MODELING

    1. MODELING AND CONTROL OF MIXED SUBSTRATES BY MIXED CULTURES
       by D. Wang, R. Wilcox and L. Evans (Mass. Inst. Tech.,
       Cambridge)

    2. MODELING THE HYDROCARBON FERMENTATIONS by L. Erickson
       (Kansas State Univ., Manhattan)

    3. MODELING CELLULOSIC FERMENTATIONS by W. Armiger (Univ. of Penna.,
       Philadelphia)

D. INTERFACING AND CONTROL

    1. EQUIPMENT AND INSTRUMENTATION REQUIREMENTS FOR CELLULOSE/CELLULASE
       PROCESSES by J.M. Nystrom (U.S.A. Laboratories, Natick)

    2. COMPUTER INTERFACING IN FERMENTATION PROCESSES
       by N. Postnikov (Inst. of Biotechnology, Moscow)

    3. PLANNING A COMPUTER COUPLED FERMENTATION PRODUCTION PLANT
       by J. D. Wilson (ABEC, Inc., Allentown)

    4. APPLICATION OF COMPUTERS TO THE ANALYSIS AND CONTROL OF
       MICROBIOLOGICAL PROCESSES by L. K. Nyiri (Fermentation
       Design, Inc., Allentown)

E. OPTIMIZATION

    1. APPROACHES TO ON-LINE OPTIMIZATION OF FERMENTATION PROCESSES
       by L. Fan (Kansas State Univ., Manhattan)

SHORT PAPERS

    1. OXYGEN AND pH ELECTRODES FOR FERMENTATION APPLICATIONS
       by M. Krebs (Instrumentation Laboratories, Inc., Lexington)

    2. COMMON PRINCIPLES OF PLANT-SCALE COMPUTER CONTROL
       by M. Beaverstock (The Foxboro Co., Foxboro)

SCHEDULE OF VISITS

OF

U.S.S.R. DELEGATION DURING CONFERENCE

MONDAY, AUGUST 11

Biochemical Engineering Laboartory, University of Pennsylvania

MONDAY, AUGUST 18

Leeds & Northrup Co., Lansdale, Pa.
Fermentation Design Co., Allentown, Pa.

TUESDAY, AUGUST 19

Foxboro Co., Foxboro, Mass.
U.S. Army Natick Development Center, Natick, Mass.

WEDNESDAY, AUGUST 20

Dept. Nutrition & Food Science,Biochemical Engineering Laboratory
Massachusetts Institute of Technology, Cambridge, Mass.

THURSDAY, AUGUST 21

Dept. Chemical Engineering, Massachusetts Institute of Technology
Cambridge, Mass.

FRIDAY, AUGUST 22

Headquarters, American Society for Microbiology, Washington, D.C.

A P P E N D I X   IV

ABSTRACTS OF CONFERENCE

I-1

ON THE PROBLEM OF MODELING

OF FERMENTATION PROCESSES AND

RESEARCH TASKS OF PROJECT 2

BY

Shamil G. Yenikeyev
Kazan Institute
for Chemical Technology
U.S.S.R.

The problem of modeling is the key point in optimal design of fermentation processes. This paper is concerned with development of a general mathematical model of multiphase fermentation processes applicable for the purpose of optimal design and scale-up. The approach is based upon decomposition of the process into a hierarchy of quasi-closed subsystems. Experimental and theoretical research tasks which are necessary in development of such a model are formulated.

I-2

THE RATIONALE AND PROBLEMS

IN THE USE OF COMPUTER-COUPLED FERMENTATION SYSTEMS

by

Arthur E. Humphrey
University of Pennsylvania
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In the past five years several companies and research laboratories have constructed and built computer-coupled fermentation systems. The coupling of a computer to a fermentation system can be beneficial for numerous reasons, these include:

1. The acquisition and reduction of data

2. Data analysis and process modeling

3. Process control and on-line process optimization

At the present time computers are mainly being used in plant facilities for data acquisition and for pre-program control of temperature and pH with the appropriate alarm functions, particularly, for anti-foam control. In pilot plant situations the computers are being used primarily for on-line data analysis and process modeling. The full potential of computers for on-line process optimization has yet to be realized. It is in this latter stage where the power of the computer can be brought to bear to aid in the rapid development and practical evolution of fermentation systems.

This paper will discuss the state of the art of computer-coupled systems, some of their limitations and problems, and propose some future objectives to be achieved with these systems.

BIOMASS CONCENTRATION MEASUREMENT

by

V. J. Babkin
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Moscow, U.S.S.R.

An important indicator in fermentation processes is
the biomass concentration. The most promising method for
biomass measurement is photometry.

This paper examines the use of a photometric method
for biomass measurement which measures the characteristic
absorption of cytochrome C in Candida yeast grown on paraffins.
Methods for compensation of problems including the influence
of medium turbidity, instability of the light source and the
coefficient of transmission of the photocell are presented.

UTILIZATION OF FLUOROMETRY, KJELDAHL, POTASSIUM, AND

OXYGEN MEASUREMENTS FOR ESTIMATING BIOMASS CONCENTRATIONS

by

Dane Zabriskie
University of Pennsylvania
Philadelphia, Penna. U.S.A.

Existing techniques for the measurement of cell mass are inadequate
in many types of fermentations with complex natural medium and medium with
high concentrations of suspended solids such as cellulose. Furthermore,
no methods are available which are readily adaptable to on-line cell mass
estimation and real-time computer analysis. This paper will discuss a
new variation of the micro-kjeldahl method for measuring organic nitrogen
which can be used to determine cell mass in a broad class of fermentations
including the cellulose fermentation. With this procedure being used as
a reliable laboratory procedure, a number of on-line estimation instruments
and techniques are being developed. These include estimates based on culture
fluorescence and component balancing. The components studied include carbon,
oxygen, and potassium. These estimates will provide valuable information
to manual or computer mediated fermentation control.

CONDUCTOMETRIC COUNTERS OF PARTICLES FOR ANALYSIS

OF MICROBIAL POPULATIONS

by

V. V. Mitjaev
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The conductometric method of particle dispersion analysis is a very useful method to·investigate microbial population structure in the production of single-cell protein. This paper presents the results from theoretical and experimental studies on this method and discusses its application to the microbiological industry. Consideration is given to problems of optimal orifice diameter, coincidence error measurement and compensation for changes in electrolyte concentration.

UTILIZATION OF THERMAL AND VISCOMETRIC METHODS FOR· BIOMASS ESTIMATIONS

by

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One of the most essential parameters in an industrial fermentation yet most difficult to measure is biomass concentration. The reason for this is that the cell concentration is usually quite high and there are frequently non-cellular solids present. Currently there are no instruments commercially available to monitor biomass concentration. For this reason we have sought to develop indirect methods of assessing the cell concentration. One of the methods examined is the measurement of heat of fermentation and its correlation with cell mass production. A dynamic calorimetric technique has been employed to measure the rate of heat evolution during the fermentation. Corrections are made for mechanical agitation. Results showing the correlation of this measurement with cell dry weight during fermentation will be presented.

A second approach currently under investigation is the use of broth viscosity to determine cell concentration. The pressure drop through a thin channel is proportional to the culture viscosity. Using state-of-the-art pressure transducers we have built a continuous viscometer to evaluate this approach. Both the theoretical rationale and experimental results will be presented.

DETERMINATION OF n-ALKANES AND INORGANIC PHOSPHATE

CONCENTRATIONS IN CULTURE MEDIA

by

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This paper is directed to the development of methods for the measurement of n-alkanes and inorganic phosphate in fermentation media. Problems in the development of these methods and the construction of a laboratory device for analysis are considered. Results from a laboratory system for analysis are presented to illustrate the utility of this approach.

Abstract: <u>Oxygen and pH Electrodes for Fermentation Applications</u>

Instrumentation Laboratory, Inc., and its partially owned subsidiary Ingold Electrodes, Inc., manufacture oxygen and pH electrodes, respectively, for a number of diverse applications which include, of course, fermentation processes.

The membrane covered oxygen electrode described originally by Clark in 1956, has been developed by IL into a rugged device capable of operation under the most adverse conditions. This electrode, called the IL 530 steam sterilizeable oxygen electrode, is of the polarographic type, having a platinum cathode and silver anode. These components together with a thermistor for automatic temperature compensation, are contained within a rugged 316 stainless steel housing. This construction as well as that of the special Teflon-silicone rubber – stainless steel mesh composite membrane, allow the electrode to be autoclaved repeatably with little or no change in calibration characteristics.

The IL 530 electrode has a 97.5% response time of ca. 20 seconds excellent linearity over the total range of oxygen levels, and exhibits virtually no sensitivity to the sample rheology (i.e. no flow sensitivity). The background current of the device is typically less than 20 ppb oxygen. This fact coupled with its excellent linearity, means that the device can be calibrated at one point, in air, even for low ppb oxygen analyses.

Ingold U.S.A. Inc., manufactures a complete line of fermentation and process control pH, reference, and ORP (Redox) electrodes along with a variety of housings both pressurizeable and non pressurizeable for use for in-line and tank measurements. The pH glasses utilized for these applications are characterized by relatively low resistance, low sodium error, and excellent stability even after steam sterilization.

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COMPUTER-AIDED FERMENTATION MONITORING AND DIAGNOSTIC

by

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The application of computers to on-line monitoring and control of fermentation processes requires the collection and interpretation of pertinent data. The number of sensors available for monitoring fermentations is limited and frequently sensors to measure the most important objective parameters i.e. cell mass, product concentration, are not available. Therefore, it is necessary to deduce the value of these parameters from available data. Parameters for which we have commercially available sensors are pH, temperature, dissolved oxygen, gaseous oxygen and carbon dioxide, and air flow rate. In addition, by means of load cells we can monitor nutrient flow rate and acid or base flow rate: using differential thermometry, we can measure heat production. From these data and the use of material and energy balances, we can now continuously assess the concentration of biomass and the conversion yield of substrate to biomass. Results from on-line monitoring of yeast fermentations will be presented to illustrate the application of this technique. Having established the ability to indirectly assess cell mass and conversion yield from readily available information, it is possible to use the on-line computer to detect deviations from optimal behavior. This is a prerequisite for the application of automatic process control. However, before this can be done effectively, we first need to know the reason for the deviation. To achieve this, we are developing diagnostic strategies to differentiate between the many possible reasons for a decline in productivity and/or cell yield. These strategies include both steady state and transient analysis of the fermentation. The application of computer-aided diagnostic analysis to the production of yeast single-cell protein production from methanol will be discussed.

MODELING AND CONTROL ON THE UTILIZATION OF MIXED-SUBSTRATES BY MIXED-CULTURES

by

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Ross P. Wilcox & Lawrence B. Evans, Department of Chemical Engineering
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The utilization of two substrates by two microorganisms has been studied experimentally in batch and continuous culture systems. The two substrates were ribose and galactose. Two yeasts, Hansenula polymorpha capable of metabolizing ribose but not galactose, and Torulopsis candida, capable of metabolizing galactose, but not ribose, were the mixed-cultures employed in these studies. It was found that in both instances, the presence of the non-metabolized substrate played an inhibitory role with respect to growth of the organism on the metabolizeable substrate. In addition to this effect of substrate interaction, it was found that population interaction also occurs. More specifically, metabolic products resulting from the mixed-cultures also inhibited the growth of the respective organisms.

The experimental results have been analyzed through mathematical modeling of these complex interactions. These computer simulations have offered some possible solutions with respect to maintaining a stable mixed population in the presence of these inhibitory interactions. For example, the Transient response of an organism to perturbations which ultimately lead to inhibitory interactions plays a predominant role with respect to overall culture stability. The use of cell-recycle was also demonstrated through computer simulation as a means of stabilizing the mixed-population to counteract the inherent culture instability. Details on the experimental results will be presented.

MODELING AND CHARACTERIZATION
OF HYDROCARBON FERMENTATIONS

BY

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The growth of microorganisms on petroleum hydrocarbons such as hexadecane and gas oil requires the transport of hydrocarbons from the oil phase to the cells and the transport of oxygen from the gas phase to the cells. The rate and efficiency of mass transfer of both hydrocarbon substrate and oxygen are keys to the economical production of single cell protein and other products. This work is concerned with measurement and characterization of cultures with two liquid phases. The identification of significant process variables which affect the mass transfer processes and of methods which may be employed for their measurement is a central theme of the present research. The prediction of mass transfer rates and growth rates from these measurements is also an integral part of the present research.

The experimental research is being carried out in shaking flasks and in a tower fermentor with motionless mixers. The tower fermentor is equipped for recirculation of the liquid phase using either natural circulation (air lift) or pumped circulation. The yeast Candida lipolytica is being grown on hexadecane and a mineral salts medium. Variables which are being measured to characterize the dispersion and mass transfer include: dissolved oxygen concentration, n-hexadecane concentration, fatty acid concentration, surface and interfacial tension, and oil drop size. Research results on the experimental measurement and characterization of cultures with two liquid phases are presented. Biochemical and physical variables which influence appreciably the rates of various transport processes are reviewed, and the measurement methodology and relationships among the variables are discussed.
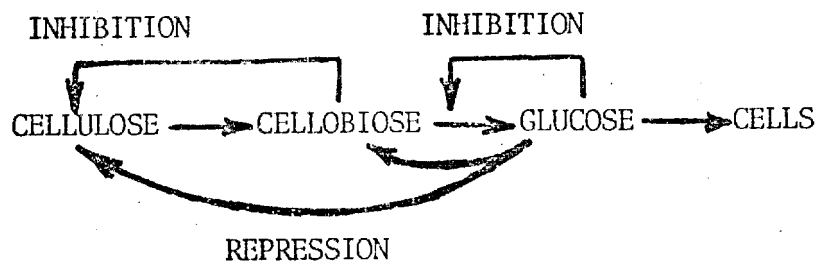
MODELING CELLULOSIC FERMENTATIONS

by

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USA

Cellulose has great potential as a substrate for the production of single cell protein. The technology of such processes has been worked out but their practicality has not been achieved. A great deal of work has been done on the pretreatment of the fiber, improvement of strains, and optimization of medium. However, very little effort has been placed on optimizing the fermentation.

At the University of Pennsylvania, we have a computer-coupled fermentation system designed for process development. The system is capable of monitoring a fermentation and at the same time simulating it on the computer. By comparison between the actual and the simulated data, it is possible to learn more about the process dynamics which may be the key to process economic improvements. In order to use this system for the cellulose fermentation, a kinetic model of the process has been developed. The model can be systematically described as follows:

INHIBITION   INHIBITION

CELLULOSE ——> CELLOBIOSE ——> GLUCOSE ——> CELLS

REPRESSION

The six linear ordinary differential equations are necessary to define the model. These are solve numerically by a fourth order Runge-kutta technique. This paper will discuss the development of the model, the determination of model parameters and constants, and the comparison between the model predictions and experimental results.

ABSTRACT

EQUIPMENT AND INSTRUMENTATION REQUIREMENTS
FOR CELLULOSE/CELLULASE PROCESSING

Bio-processes utilizing insoluble substrates present the bio-engineer with a variety of problems, amoung them the handling and transport of solids and slurries, effective oxygen transfer and estimation of cell mass in the presence of insolubles. The production of cellulase enzymes in submerged culture and the continuous enzymatic hydrolysis of waste cellulose are two such troublsome processes. The selection of equipment, instrumentation, and operating parameters are all of equal importance as is the development of appropriate systems and models for data reduction.

Pilot plant investigation of the _Trichoderma viride_ fermentation for cellulase production and cell free enzymatic hydrolysis of waste paper are presently carried out in highly instrumented fermentors and reactors. The vessel designs and instrumentation allow for performance of the most modern fermentation techniques and processing strategies. This has led to the establishment of reproducable fermentation profiles and models for optimum inoculum preparation and product yields.

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COMPUTER INTERFACING IN FERMENTATION PROCESSES

by

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Three special electronic devices for computer interfacing
have been described. At the present time, two prototype
devices have been built. The first is for the conductometric
sensor. The second prototype is for the hydrocarbon concentra-
tion sensor. The operation of the third device, which is used
for data compression, has been simulated on a computer with
good results. The block diagrams and time plots of signals
are presented.

PLANNING A COMPUTER COUPLED FERMENTATION

PRODUCTION PLANT

by

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The ultimate test of a plant design is the unit cost of the product produced. Among the current factors influencing fermentation costs are fluctuating and upward trending raw materials prices, much higher energy costs and increased price competition as some products shift from proprietary to generic status. The commercial development of new processes as well as expanded production capacity for existing ones will likely depend on attractive investment return based on anticipated lower unit costs. This paper will discuss the role of the computer in modern fermentation plant operation and its potential for improving plant efficiency.

APPLICATION OF COMPUTERS

TO

ANALYSIS AND INTERACTIVE CONTROL

· OF

MICROBIOLOGICAL PROCESS*

BY

NYIRI, L.K., G.M. TOTH, C.S. KRISHNASWAMI AND D.V. PARMENTER

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ABSTRACT

Digital computers have proven to be useful for acquisition of process variables and definition of process (activity) indicators. This made it possible to perform pulse testing of cultures, altering environmental variables and observing the responses in changes of the culture's rheological and physiological conditions. Analysis of data resulted in development of control technique which performs the control of individual environmental variables on the basis of the physiological condition of the culture. The environmental variables are changed in an interactive way, taking the previously defined correlations between the cells and their environment into consideration. The application of such a control technique in molasses based SCP production resulted in enhanced yield and productivity of C. utilis cell mass.

PRINCIPLES OF ON-LINE DYNAMIC
OPTIMIZATION IN FERMENTATION SYSTEMS

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This paper discusses the principle and application of the GMDH
algorithm (the Group Method of Data Handling) to on-line optimization
in fermentation systems. The GMDH algorithm is based on the principle
of heuristic self-organization and is a new technique for mathematical
simulation that permits a gradual increase in the complexity of the
models as long as this results in simulation accuracy (e.g., in the
least mean squares sense). Although there are many variants to the
GMDH algorithm, the most common versions implement a truncated
Kolmogorov-Gabor polynomial in the form of a multilayer system of the
perceptron type (a neuromine network of predictive components). Data
are partitioned into a training subset and a testing subset; the former
is used in conjunction with a newly developed iterative random search
procedure to obtain coefficient values that are globally optimum
while the latter is used to determine the optimum degree of complexity
of the model.

Finally, an example will be used to illustrate the advantages of
the GMDH algorithm, which include a high accuracy of prediction and
fast learning with a limited amount of data.